Commonwealth of Pennsylvania.

BULLETIN No. 7

DEPARTMENT OF FISHERIES

OBSERVATIONS AND EXPERIMENTS ON THE SO-CALLED THYROID CARCINOMA OF BROOK TROUT (SALVELINUS FONTINALIS) AND ITS RELATION TO ORDINARY GOITRE.

By DAVID MARINE, M. D.
AND
C. H. LENHART, M. D., Cleveland, Ohio.

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, in Conjunction with the Pennsylvania State Department of Fisheries.

W. E. MEEHAN,
Commissioner of Fisheries.

ISSUED MARCH 1, 1910.

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INTRODUCTORY NOTE.

A disease commonly called throat tumor has long been known to fish culturists. It is a cone shaped protuberance which appears usually in the throat of trout, particularly the charr. The cause is unknown but it has not generally been believed to be particularly serious, and many owners of trout hatcheries view its existence among their fish with entire indifference. The "tumor" is not known to occur among wild trout, but only among those held in captivity. Its existence was regarded as due sometimes to bruising, often to dirty ponds, and occasionally to overfeeding with unnatural foods. Within the last year or two, some who give specially close study to the subject of fish culture and diseases of fish began to regard the disease more seriously, the more especially since nearly every hatchery in the country, whether under Federal, State or private control, seemed to be to a greater or less extent affected. Among others, I gave the subject much anxious thought, although all but one of the State hatcheries seemed comparatively free. Early last year I received a communication from Dr. David Marine, one of the faculty of the Western Reserve University, Cleveland, Ohio, inquiring what disease, if any, exists in the State or private establishments. I wrote him concerning the "throat tumor," and it was this particularly, it seems, which he was desirous of finding. It appears that suspicions had been aroused with respect to the disease which instead of a simple goitre, it was believed by many to be a true cancer.

After further correspondence, the Western Reserve University agreed to co-operate with the Department of Fisheries of this State in making an investigation of the disease, the result to be published as a State bulletin. The work in Pennsylvania was undertaken by Dr. Marine in collaboration with Dr. Lenhart. Investigations both in America and Europe have regarded the disease as cancerous in nature. I regret to say that while the findings of Dr. Marine and Dr. Lenhart at the present stage of the investigation, do not warrant a declaration that it is true cancer, on the other hand indications point to the fact that in its early stages, it should be classed as a severe edemic goitre. The possibility of its development later into true cancer is not denied.

The United States Bureau of Fisheries, with the co-operation of the Forestry, Fish and Game Department of the State of New York, has been carrying on extensive investigations at the Buffalo laboratory, under the direction of Dr. Harvey Gaylord, on this disease at the same time as the Western Reserve University and the Depart-

ment of Fisheries of Pennsylvania. I do not know how far the work has progressed under the direction of the National Government, but so far as we know they have published no report of experiments.

It is interesting to note that Dr. Marine, as far as his investigations have gone, has found that where trout affected with enlargement of the thyroid were placed in an open stream they recovered. In this he is borne out by a report made to me by the owners of a private hatchery in this State, in which a few years ago the disease was exceedingly prevalent. Acting under my advice the ponds were thoroughly cleansed, an increased water supply introduced, and the amount of food reduced. A year later the owners reported that the disease had entirely disappeared. I have not personally verified this statement, but I have no reason to doubt it. While the situation is undoubtedly grave for fish culturists, especially for those who raise trout for the market, there is not the slightest cause for alarm among the public, and there is no need for the people to discard fish, especially trout, as an article of food, for fear lest they might develop cancer.

This paper being published as an official bulletin of the State, is strictly a scientific document and it was thought best it should be read before a scientific body before printing, and this was done February 11, 1910, before the Cleveland Academy of Medicine, where its title and substance is a matter of record. The bulletin now published is only preliminary to another which will follow further investigation on this particular subject. It is undoubtedly one of the most important economic questions that has arisen in the fish cultural world since fish culture has been a science.

W. E. MEEHAN,
Commissioner of Fisheries.

INTRODUCTION.

The disease known as thyroid carcinoma is characterized by the abnormal and excessive growth of thyroid tissue leading to the formation of visible tumors which may appear ventrally in the region below the base of the tongue or dorsally in the floor of the mouth and pharnyx between the first and third gill arches. Secondary growths are frequently to be seen in the gills and at the anterior extremity of the lower jaws. (See figure 1.)

So far as is definitely known at present this severe degree of the disease is confined to carnivorous fish reared in captivity. Its spread has been parallel with the recent great development of fish culture and the artificial rearing of large numbers of game fish—especially the *trout* and *salmon*.

According to Pick, (Berlin Klin. Wochenschr, 1905, XLII, 1435) this disease was first positively recognized as carcinoma of the thyroid by Marianne Plehn, (Allgem. Fischerei Zeitg. 1902, 117). The disease had been definitely known since the seventies, but its exact nature or even its thyroid origin was disputed. (Pick loc. cit.) At Present the disease prevails in all countries where trout and salmon are artificially reared.

Up to the present time no systematic studies have been recorded apart from the histological observations above referred to. The observations and experiments here reported were undertaken to ascertain whether there was any relation between ordinary goitre as seen in fish and animals, and this cancerous affection of the thyroid. Pick, from his purely anatomical studies, has suggested that this cancerous affection might be only the terminal stage of an earlier simple goitrous condition.

GENERAL OBSERVATIONS.

Our observations and experiments were made during October and November, 1909, at a large private fish hatchery in the mountains of Pennsylvania.

The arrangement of the houses and retaining tanks is quite simple. This is shown in Fig. 2. The tanks are arranged in a single series down the course of the brook. The water supply is likewise simple. There is but the single spring supplying the upper six houses while the lower five receive the water that has passed through the upper six together with that from a second small spring and a six-inch pipe line from a large stream about a quarter of a mile to the left. On account of the increased water supply and for the clearer understanding of certain observations to be mentioned later, it is best to divide the series of tanks into an upper and a lower division. Between the upper and lower divisions of houses, (that is between houses VI and VII) the water follows the original brook for a distance of about a quarter of a mile. Then it is again collected by means of a dam and enters the lower division of houses with the additions to its volume above mentioned. The water coming from the spring above all houses to the first house—a distance of about fifty yards—is also allowed to follow its natural bed. Apart from these two exceptions the water is carried in covered raceways made of lumber.

The distribution of the fish according to *age* is important and accidentally fortunate from our point of view inasmuch as the three pairs of tanks likely to show the greatest variations in the condition of the thyroids contained fish of the same age, viz: one and two-thirds years (1 2-3).

Taking up the arrangement according to age (see Fig. 2) it is seen that houses I, II, III, and IV contain eight months' old fish; houses V, VI, VII and VIII contain one and two-thirds years (1 2-3) old fish and houses IX, X and the first two pairs of tanks in house XI contain two and two-thirds years (2 2-3) old fish. The last pair of tanks in house XI contains one and two-thirds years (1 2-3) old fish. As already pointed out the three most important locations as regards the effect of water supply and water pollution are houses VI, VII and the last pair of tanks of house XI containing fish of the same age viz: one and two-thirds years (1 2-3).

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*We shall use the word "tank" throughout this paper although they are usually designated "ponds" by fish culturists. These tanks are made by excavating the soil and their walls shored up either with plank or concrete. The inclosed space may be of any dimensions desired but at the hatchery in question they averaged six to seven feet wide by twelve to sixteen feet long and two to three feet deep. The bottoms are sometimes made of plank, sometimes of concrete but more commonly of sand or gravel.

It occurred to us that it might be interesting to ascertain the number of fish with visible tumors in a given number of fish at these locations. Accordingly we examined series of two hundred and ten fish from each of these locations viz: the last pair of tanks in house XI, the uppermost pair of tanks in house VII and the last pair of tanks in house VI. The fish were collected in a seine and two hundred and ten counted without selection on two successive days.

House XI.....tank 36.....210 fish.....3 visible tumors.
House XI.....tank 36.....210 fish.....4 visible tumors.
House VII.....tank 20.....210 fish.....2 visible tumors.
House VII.....tank 20.....210 fish.....3 visible tumors.
House VI.....tank 19.....210 fish.....6 visible tumors.
House VI.....tank 19.....210 fish.....8 visible tumors.

The result was surprising in that the uppermost tank shows a much larger percentage of visible tumors. Tanks 36 and 20 are practically the same, the percentage being perhaps slightly less in tank 20. To explain the great differences between tanks 19 and 20 the possibility of one tank having a larger number of fish was raised and the caretaker told us that the same number of fish was placed in each. From our examinations we would agree that there was no very great difference in the number in the two tanks. A more important and direct factor lies in the water supply and is pollution. Tank 20 receives about three times the amount of water that tank 19 does and has the two added advantages that the two additional supplies are fresh from the soil and also the water from tank 19 has traversed its natural brook bed for a distance of nearly a quarter of a mile before again entering the tanks. Thus as regards volume and pollution of the water, tank 19 is in a very bad position. We are inclined to attach considerable importance to this decreased water supply and increased pollution as explaining the more severe thyroid disease in tank 19 and also the increased water supply with its factors of dilution and perhaps some reduction in the products of decomposition of food and excreta as explaining the greatly improved state of the fish in tank 20.

This same difference is distinctly noticeable in the fish without visible tumor as will be referred to in the discussion of the "tank series" which follows.

OBSERVATIONS ON THE HISTOLOGICAL STATE OF THE THYROID GLANDS IN A SERIES OF FISH REPRESENTING SPECIMENS FROM ALL TANKS.

Following up the lead gained from enumerating the number of visible tumors in a given series of fish of the same age from crucial points in the line of tanks, we collected specimens without choice from all the tanks, also specimens of different ages from the raceways

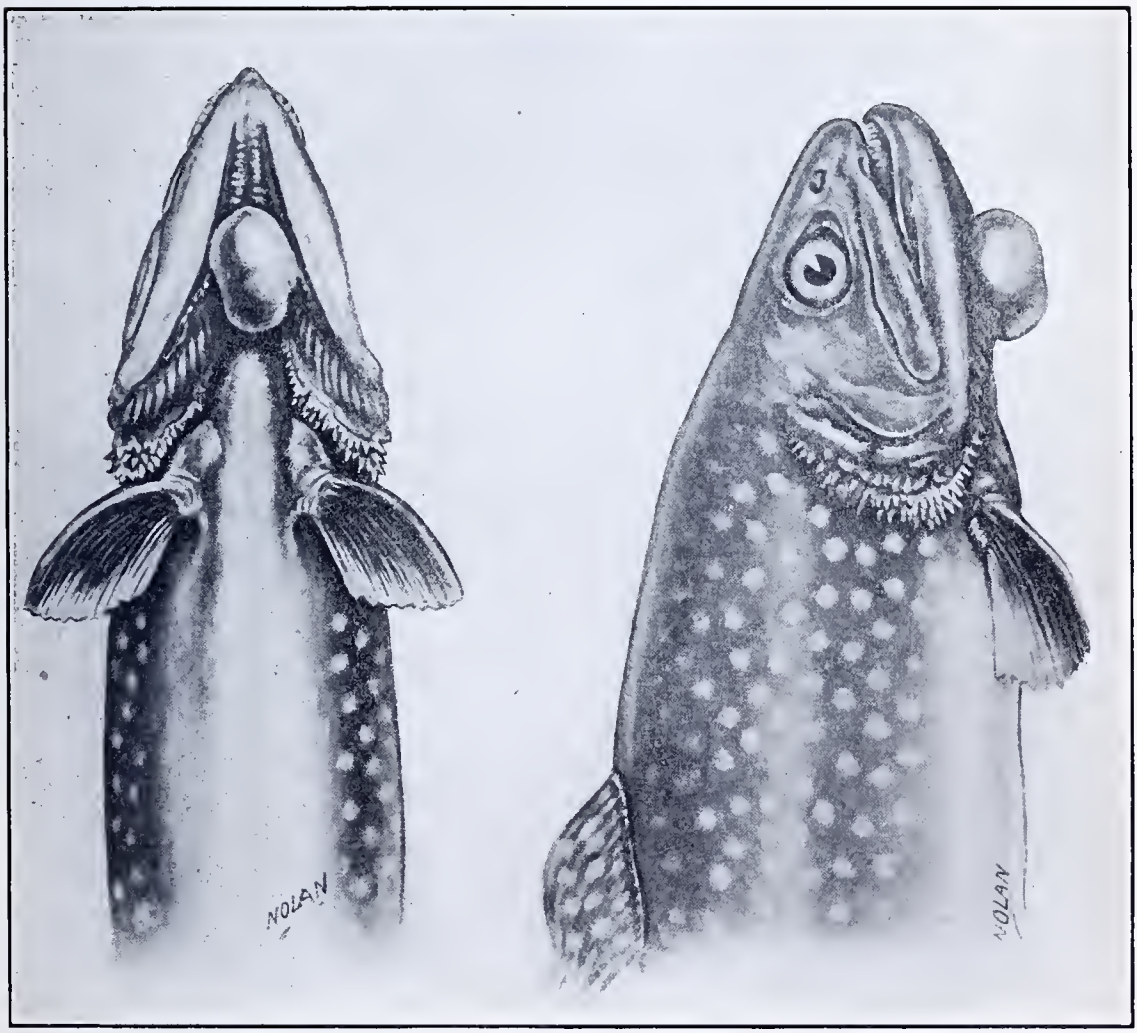

FIG. 1.

above and *below* all houses, and lastly, specimens of the larger three and two-third years' old fish from the trout stream near by.* We have tabulated the histological condition of the thyroids and have arranged them in order from above downward. (See table No. 1).

Beginning with the fish taken from the raceway above all houses: The thyroids were found to be normal in each instance irrespective of the age of the fish. (See Fig. 2.) These fish living in the raceway, we were told, had escaped from the tanks as fry when they were being transferred from the troughs in the hatch house to the tanks, and therefore had spent their entire lives, since removal from the troughs as fry, in this small stream of water of about forty yards in length. They were not fed or cared for in any way.

Passing on to the tanks, it is seen that tanks 1-13 contain two-thirds year old fish, that is, fish hatched in the *winter* and transferred to the tanks as fry in the late spring. They were all healthy large fish. Histological examination revealed the fact that all these fish had marked thyroid changes although none had visible ventral tumors. This thyroid proliferation had, in most cases, invaded the muscle, bone, cartilage and filled in all the areolar tissues beneath the epithelium of the floor of the pharynx. There was very little difference in the extent of the thyroid proliferation throughout the 13 tanks, although the specimens taken from tanks 10-13 showed the most marked proliferation.

The one and two-thirds (1 2-3) years old fish are contained in tanks 14-25 inclusive. Here very great differences are to be noted in the thyroid condition between the fish from houses V and VI of the upper division and houses VII and VIII of the lower division. As pointed out above, the number of visible tumors was very much greater in the upper houses (V and VI) than in the lower houses (VII and VIII). So also the histological appearance of the thyroids is very much worse in houses V and VI than in VII and VIII. In the fish of the upper houses the epithelial proliferation has extended to all the structures beneath the floor of the pharynx and even out into the gill arches. There is rarely any stainable colloid to be seen and the epithelium is everywhere columnar. In other words the epithelial proliferation, started during their first year of life, has progressively increased during this their second year of life. On the other hand the thyroids of fish from houses VII and VIII contain moderate amounts of stainable colloid. The epithelium is in general low columnar and the extent of the proliferation has not progressed beyond the stage reached during the first year of life while occupying tanks 1-13. In other words some agency has been acting which has successfully prevented further hyperplasia and even

*The plan followed at the club has been that each April the three year old fish are transferred to the trout stream from whence they are taken by the club members with hook and line. It was some of these fish that we examined.

started the process of involution (reversion) of the thyroid hyperplasia to the colloid state. As above mentioned the most patent factor seems to be the greatly increased water supply with its added factors of increased purity and dilution of the products of decomposition and putrefaction.

Passing now to the two and two-thirds (2 2-3) years' old fish it is seen that they occupy tanks 26-35 inclusive. In all of these fish the thyroids have undergone very extensive changes and added factors of histological complexity have to be taken into consideration by reason of their longer lives; the fact that they have been moved twice (that is, occupying tanks 1-13 during their first year and tanks 14-25 during their second year): the fact that it is impossible to determine which of the upper tanks they had previously occupied—all these factors undoubtedly enter into the explanation and interpretation of the more varied histological appearance of the glands, just as similar factors are known to modify mammalian thyroid hyperplasias. In most of the specimens examined there is stainable colloid present. The extent of the proliferation and the epithelial types are quite variable. In general the epithelium is of the columnar type as opposed to the high columnar type noted in houses I-VI. Another factor of perhaps great importance is the noteworthy increase in the fibrous stroma, both relative and absolute, throughout the area of thyroid invasion. While it is impossible to speak of this group in terms specifically applicable to all the specimens, the general impression gained is that the proliferation is less active and the tendency toward the arrest of the growth is more evident. The prominence of the stroma, the presence of colloid and the lessened height of the epithelium are, we believe, evidences of this tendency toward arrest.

Next in order several specimens taken from the tail-race below all houses were examined. Here, as in the raceway above all houses, the fish are left to care for themselves. Several specimens of two thirds (2-3) year old fish were taken. These fish, the caretaker told us, were in all probability specimens of the winter hatch which could not be taken care of in the tanks and were planted in the trout stream and had come into the tail-race to avoid the bigger fish of the larger stream. The thyroids of these fish were slightly larger than in normal fish of the same age. Their colloid contents and epithelium were normal. We took this to mean that even though living in the polluted water below all the houses, but in the wild state, they were able to recover from whatever active thyroid hyperplasia they had at the time of planting and also to maintain normal types of thyroid while living in such an environment. We also took specimens of three and two-thirds (3 2-3) years old fish both from the tail-race (fish that had come up from the larger stream to spawn)

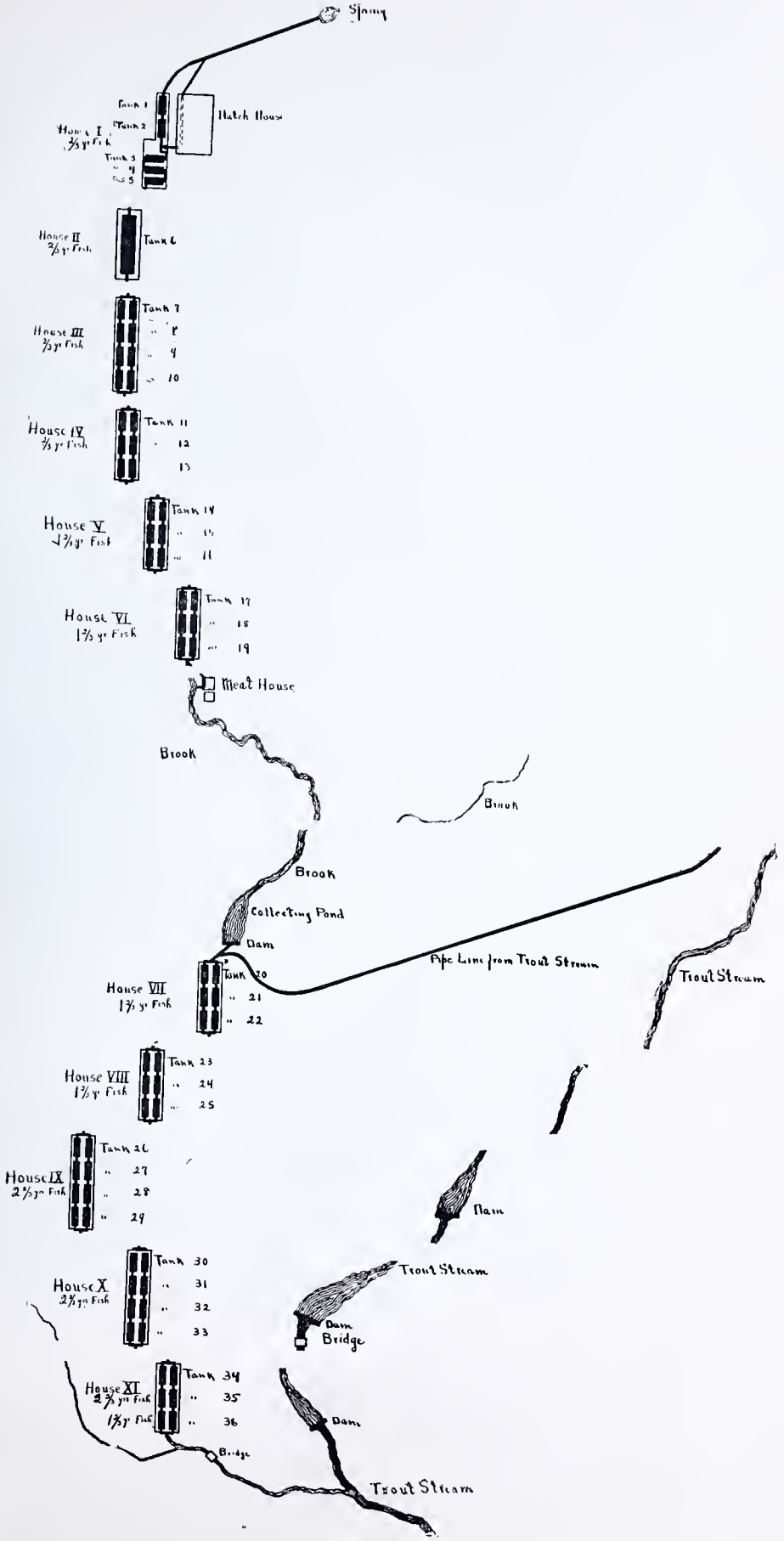
Spring
Hatch House
House I
House II
House III
House IV
House V
House VI
Meat House
Brook
Brook
Brook
Collecting Pond
Dam
Pipe Line from Trout Stream
Trout Stream
House VII
House VIII
House IX
Dam
Trout Stream
Dam
Bridge
House X
House XI
Dam
Bridge
Trout Stream

FIG. 2.

and from the main stream above the entrance of the tail-race stream. In these specimens we observed perhaps the most important changes thus far recorded viz: that in all these fish the thyroids had returned to the pure colloid state, although the entire sub-pharyngeal space was filled with thyroid follicles including the bone, cartilage and striped muscle. Here one sees written plainly the life history of these fish thyroids, viz: that the extreme thyroid proliferation and invasion of all the structures beneath the pharyngeal mucosa occurred during their stay in the tanks, and that during their 5-6 months stay in the open stream they had completely recovered from the disease and their thyroids had returned to as nearly their normal states as was possible for such hyperplastic thyroids.

To sum up, it has been seen that the fish living in the raceway above all houses and which had never been confined in the tanks, maintain normal thyroids throughout their lives. Then, beginning with the series of tanks it was shown that the eight months old fish are markedly affected even in the uppermost of all the tanks and that there is a gradual increase in the degree of thyroid proliferation which reaches its greatest extent and severity in the last house (VI) of the upper division. Beginning with house VII there is a marked improvement and lessening of the active thyroid proliferation coincident with the greatly increased water supply and the probable purification of the water from the upper division in its passage for a quarter of a mile along the original bed of the brook. This improvement cannot be followed definitely throughout the remaining houses of the lower division for the reason that the two and two-thirds (2 2-3) years old fish are housed here, but the general impression is that this improvement is maintained throughout the remaining tanks to a gradually lessened extent. Lastly the examination of fish from the tail-race, living wild but in the polluted water, has shown that their thyroids are normal in type. Also examinations of older fish obtained both from the tail-race and the larger stream above the entrance of the tail-race into it have shown that although the thyroid tissue has invaded all the structures beneath the pharyngeal mucosa at some past period in their lives nevertheless their stay of five to six months in a natural environment has affected a complete natural relief from the exciting cause or causes of the thyroid proliferation and that the thyroids have assumed their resting or colloid state.

EFFECT OF IODIN ON THE THYROID HYPERPLASIA.

This experiment was carried out as follows: An ordinary trough with inside measurements of 120 by 20 by 18 inches and a capacity of 25 cubic feet was placed in house XI and arranged so that water could be supplied at a uniform rate from the main stream supplying the

tanks. The water supply was regulated by means of a shunt and so arranged that in the fall of ten inches the column was broken to allow aeration. This stream was adjusted so that it filled the trough in four hours, thus making it possible for the complete replacement of the water six times every twenty-four hours. This rate of flow was kept the same throughout the experiment.

On October ninth, six healthy looking and clinically tumor free fish were taken from house XI and tank 36, killed at once and kept for *controls*. At the same time fifteen healthy looking and clinically tumor free fish were taken from the same tank and placed in the trough. Iodin in the form of Lugol's solution (KI=3 gms I=1gm. water=300 c. c.) was used. Two c. c. of this solution were adden to the water of the trough each morning and evening. One fish was taken every second day for histological examinations (October 11, 13, 15, etc.) The result of the histological examinations follows:

TABLE 2—CONTROL FISH. IODIN EXPERIMENT.

No.	Fish Age.	Histological Condition of Thyroids.			Classification.
		Colloid.	Infiltration.	Epithelium.	
1	1⅔ years,	Reduced,	Bone, muscle and all the areolar tissues beneath the pharyngeal mucosa.	Columnar,	Moderate glandular hyperplasia.
2	1⅔ years,	Reduced,	Bone, muscle and areolar tissues,	High Columnar,	Moderate glandular hyperplasia.
3	1⅔ years,	Practically absent,	Extensive growth in bone, muscle cartilage and all the areolar tissues beneath pharyngeal mucosa.	High Columnar,	Marked glandular hyperplasia.
4	1⅔ years,	Reduced,	General growth throughout areolar tissue. No def. invasion of bone and muscle.	Columnar,	Moderate glandular hyperplasia.
5	1⅔ years,	Reduced,	Bone, muscle and areolar tissues beneath pharyngeal mucosa.	Columnar,	Moderate glandular hyperplasia.
6	1⅔ years,	Practically absent,	Bone, muscle cartilage and all areolar tissues.	High Columnar,	Moderate-marked glandular hyperplasia.

The six specimens (table 2) taken as controls show quite uniform thyroid changes. In all the thyroid proliferation extended into the bone, the striped muscle and occupied the entire fatty-areolar space beneath the floor or pharynx. The colloid was reduced in all and practically absent in two. The epithelium was columnar in all and the degree of proliferation ranged between *moderate* and *marked* glandular hyperplasia, using the same standards as adopted for the mammalian thyroid hyperplasias.

The fifteen specimens recorded in table 3 had been exposed to the iodin containing water for periods ranging from two to twenty-two days. The experiment was terminated on the twenty-second day and the remaining five specimens taken.

It can be seen that little or no change is to be noted until the eighth to the tenth days. Then the increase of colloid and the changes in the epithelium toward cubical are evident. The process is gradual, reaching the pure colloid state on the twentieth day in one case, although in two of the specimens taken on the twenty-second day the return to colloid was not quite complete. This is due in all probability to the somewhat more extensive proliferation in these specimens at the beginning of the experiment.

When one compares the rapidity of the reversion process in mammals with fish it is seen that mammals react much more quickly with iodin. The question arises would fish glands react more quickly if iodin could be given direct (by mouth or subcutaneously) as is done with dogs. Perhaps to a slight extent the process could be hastened but it seems more likely that in these cold blooded animals the tissue changes are normally slower. This same iodin effect was obtained with the less extensive hyperplastic (goitrous) thyroids of the Lake Erie pike. (Johns Hopkins Hosp. Bul. 1910, April.)

Since fish thyroid hyperplasias are similar histologically to those of mammals, and since they react in the same way with iodin, the propriety of calling this fish thyroid hyperplasia, *carcinoma,* is very doubtful. It has thus been designated on account of the histological evidence of invasion of bone, muscle, etc. As was pointed out above this is quite valueless in the case of the fish thyroid where the gland is not encapsulated and follicles of even the normal thyroid may be imbedded in bone and muscle. When such a thyroid undergoes hyperplasia, histological preparations would give quite definite pictures of invasion. The biological test (the reaction with iodin) is the only one that can be relied upon in the case of the thyroid not only for fish but for many mammalian types of hyperplasia. We feel certain that in human pathology many cases were diognosed as cancer that were not cancer. We have never seen the iodin affect true cancer of man or dogs. As to whether there is not an end stage of this hyperplasia that is true cancer is a separate

question. That is quite possible and is also in conformity with our present ideas of cancer development. Certain it is that this tumor of the brook trout thyroid is not cancer in the stages in which it is usually seen and studied, although histologically it gives the actual appearance of invasion and destruction of adjacent tissues.

INOCULATION EXPERIMENTS.

On October tenth, fifteen one and two-thirds years old fish from house XI, tank *36,* were inoculated. Seven were inoculated with a tumor from a one and two-thirds years old fish and the remaining eight with a tumor from a two and two-thirds (2 2-3) years old fish. Histologically both tumors were identical. They were typical examples of hyperplasia with very scanty colloid substance, marked infoldings and plications and the epithelial tissue had invaded the bone and muscle of the sub-pharyngeal region. There were also small tumor bulgings beneath the epithelium of the floor of the pharynx. Both fish had secondary thyroid growths at the tips of their lower jaws.

We used the Bashford and Murray method of inoculating small cylinders of the tumor in a trocar and shoved out by means of a stylette. The inoculations were made subcutaneously just behind the shoulder girdle on the left side. The small skin wound was bathed in Sat. sol. NaCl to prevent fungus growth at the site. Specimens were taken every third day. The inoculation area and the thyroid were kept for histological examinations.

The first specimen was taken October thirteenth and the last November twenty-fourth. Specimens taken October thirteenth and October sixteenth show clumps of thyroid epithelial cells at the base of the trocar wound. In none of the specimens taken later could we make out any cells comparable to thyroid cells. There was no evidence of local infection in any of the specimens. The specimens taken from October nineteenth to November twenty-fourth show at the inoculation site only the evidence of slight haemorrhage with scar formation. The thyroids of all the fish used were macroscopically not enlarged but histologically there was extensive proliferation and invasion of the bone and muscle in all. The experiment is not tabulated for the reason that the results were entirely negative. In spite of our negative results we are rather inclined to believe the tissues can be transplanted after we have become more familiar with the physiological requirements. The site we chose for inoculation was purely for convenience. We have never observed secondary growths except in the gills and at the tip of the lower jaw and it would seem that these locations would be more favorable for the growth of transplants. However there is still doubt whether these growths in the gills and at the tip of the lower lip are actual metastases.

ANATOMICAL DESCRIPTIONS OF THE THYROID CHANGES.

As a result of moderately extensive histological examinations of the thyroids of sea trout, sea bass, lake trout, brook trout, pike, bass, herring, white fish, carp fish and gold fish, we have come to the following conclusions regarding the normal thyroid in these bony fish. (a) The *normal thyroid* (see fig. 3) in these several varieties of fish, representing widely different areas throughout the United States, shows only minor individual differences. It is also strikingly like the normal thyroid of mammals as regards the histological appearance of the thyroid unit—the alveolus or follicle. These alveoli are loosely distributed, separate or in small collections in the areolar tissue about the ventral aorata between the first and third gill segments. Scattered follicles extend outward for slight distances on the second and third gill arch arteries. *The gland is not encapsulated* as in reptiles, amphibia and all higher animals. This fact is of the greatest importance in the interpretation of the pathological conditions in-as-much as isolated follicles are often seen lodged in bony angles or in the fascia of striped muscle quite distant from the main thyroid area. The main mass of thyroid tissue lies in the second gill segment and scattered alveoli in the areolar tissue extend antero-posteriorly and laterally from this central mass. (See fig. 4).

Histologically the gland is very vascular. Each alveolus is surrounded as in the mammalian gland with a rich capillary network. The alveoli are rounded and show the same variations in size as is seen in higher animals. While the size of the alveoli of course varies with the size and age of the fish as in other animals, the majority of alveoli would be included in measurements of 0.1-0.5 m m. in diameter. The lining epithelium is low cubical regularly arranged in a single layer with small darkly staining nuclei. The colloid completely fills the alveolus, stains uniformly with eosin and comes in direct contact with the lining epithelium. (See fig. 5.) In its general histological appearance therefore the normal thyroid of these bony fish is similar to that of mammals in all its essential characteristics except that there is no capsule.

(b) *Hyperplasias.* (See figs. *6 and 7)* In the development of goitre the thyroid undergoes certain characteristic changes which are designated by the term "Hyperplasia." These changes in the fish thyroid are similar in all their essential features to the hyperplasias in mammals as typified by the spontaneous or induced *(following partial removal)* hyperplasias of dogs or the hyperplasia of exophthalmic goitre in man. As in the higher animals so with fish, there are all degrees of active thyroid hyperplasia ranging from the slightest departure from normal (hypertrophy) to the marked glandular hyperplasias. As will be reported elsewhere this active hyperplasia of fish thyroids in a mild form occurs in certain Lake

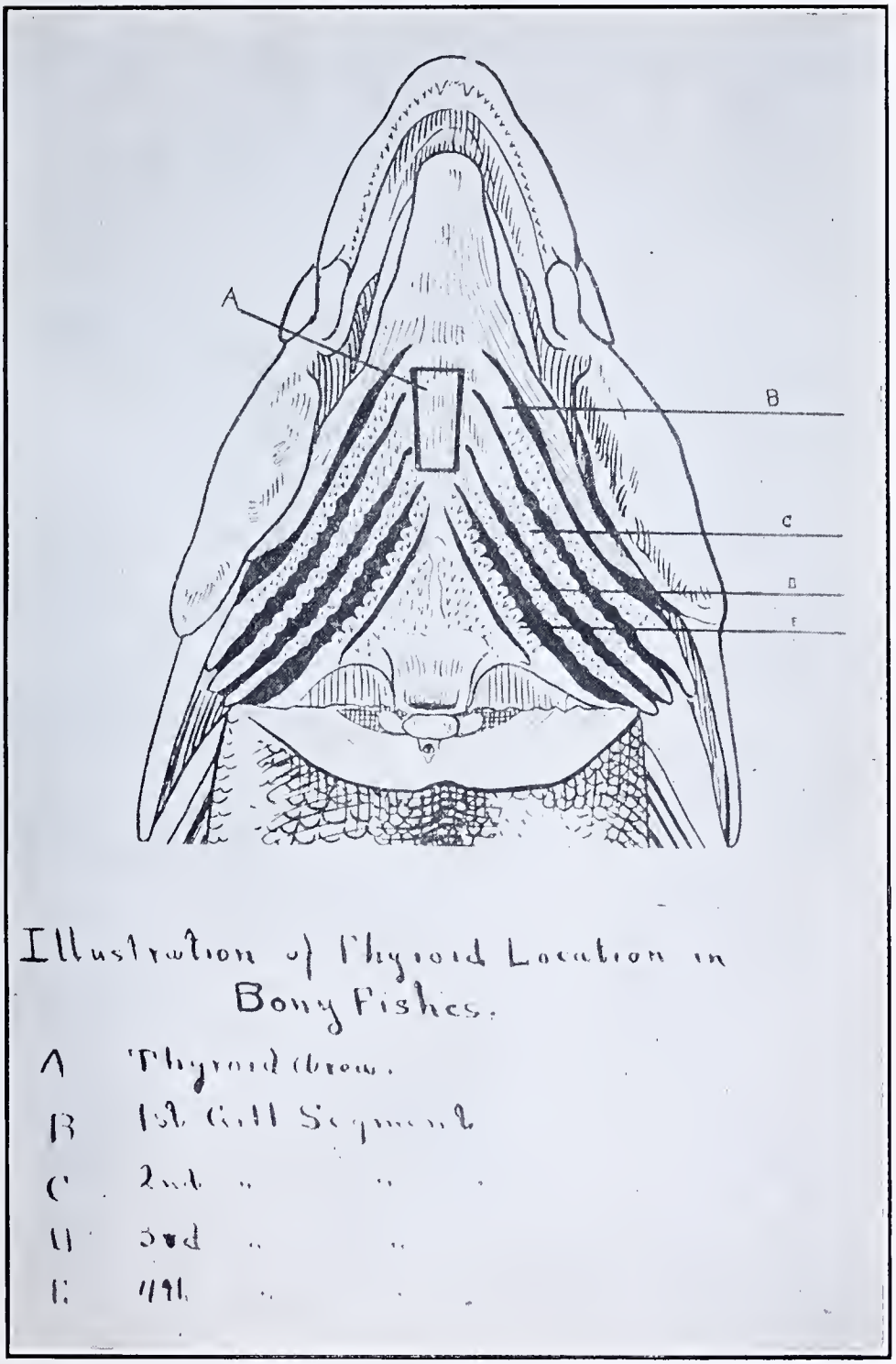
A
B
C
D
E
Illustration of Thyroid Location in
Bony Fishes.
A Thyroid Gland.
B 1st Gill Segment
C 2nd " "
D 3rd " "
E 4th " "

FIG. 3.

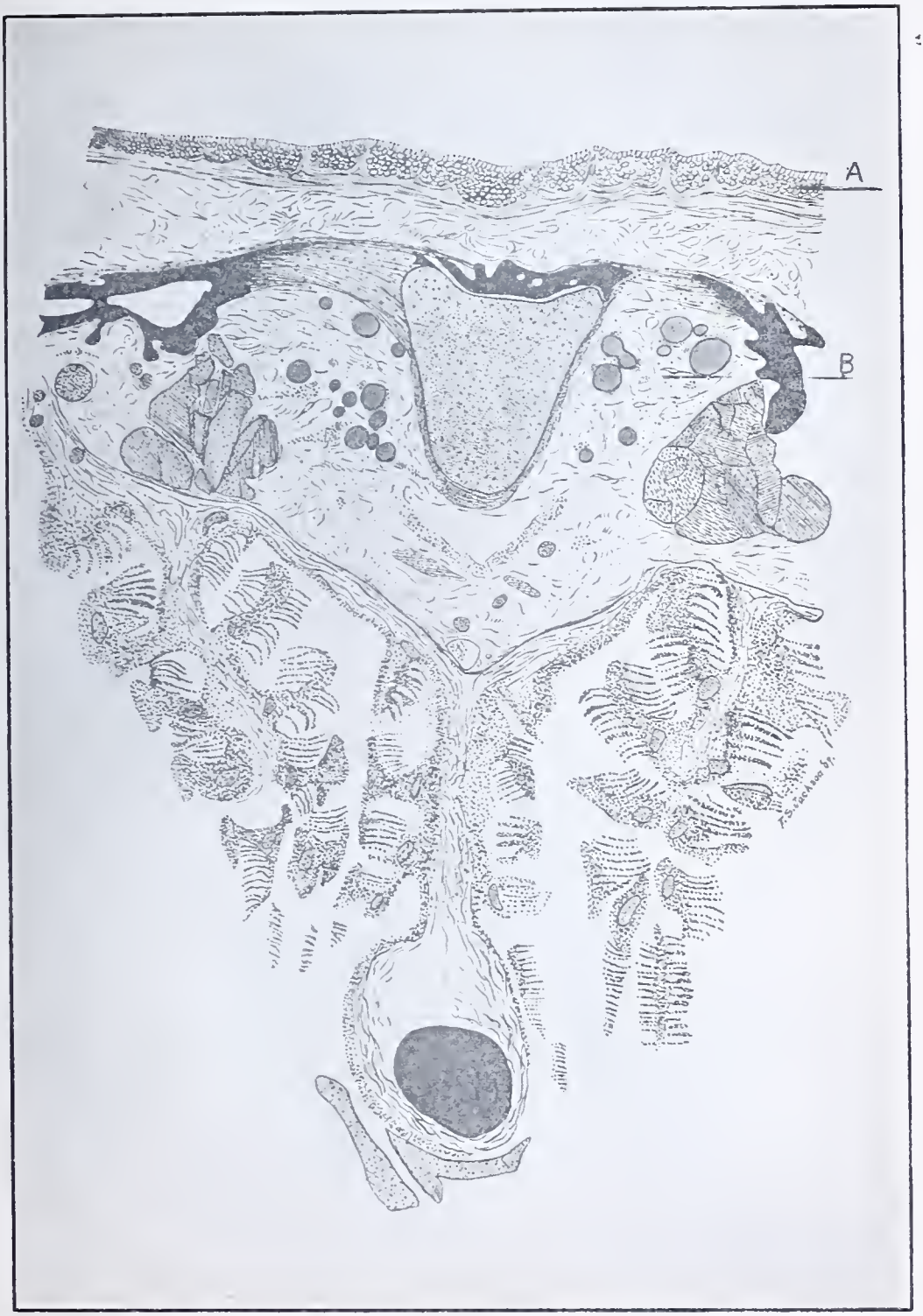

FIG. 4.

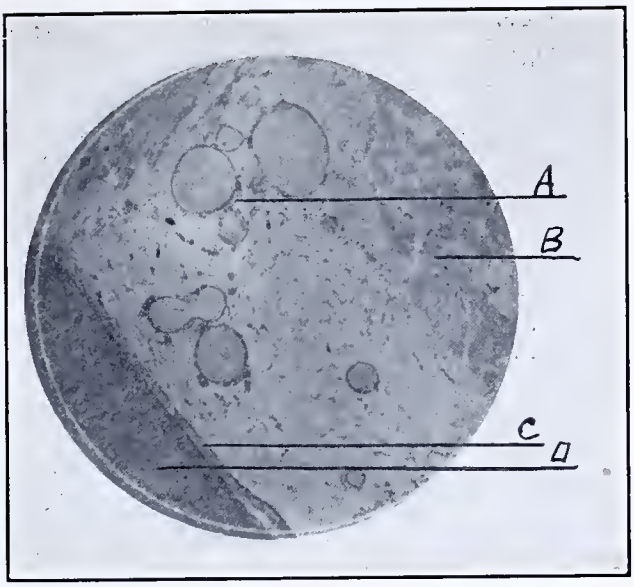

FIG. 5.

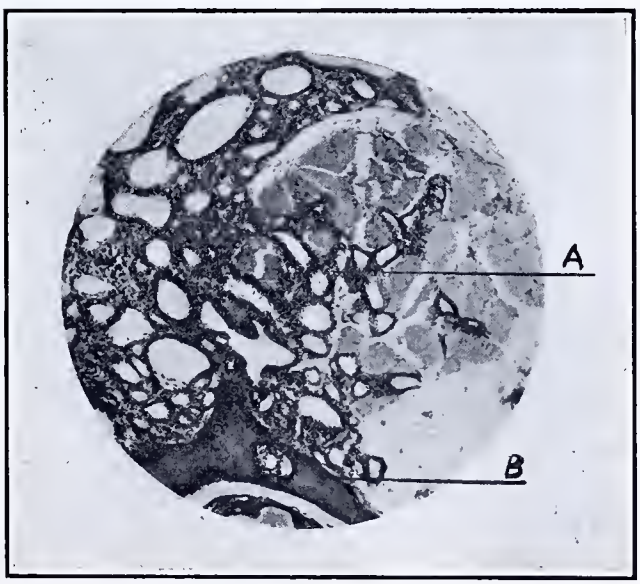

FIG. 6.

Erie fish in their wild state, but the severer grades going on to visible tumor formation have been thus far observed only in the carniverous fish (trout, salmon) reared in captivity.

The most striking histological changes are the same as in mammalian thyroids and are seen in the *blood vessels,* the *stainable colloid* and the *epithelium.* The capillaries and larger vessels of the stroma are dilated and hypertrophied. The stainable colloid is decreased, but slightly in the early stages of hypertrophy while it may be entirely absent in the marked degree of hyperplasia. The epithelium lining the alveoli varies from cubical in the mild degrees of hypertrophy to high columnar in the marked degrees of hyperplasia. The infoldings and plications of the lining epithelium are present in all the more marked degrees but perhaps to a less extent than in similar degrees of hyperplasia in dogs. This may perhaps be explained by the absence of the restraint normally exerted by the gland capsule of higher animals. It is thus seen that the hyperplasias of fish are similar in all their essential characteristics (anatomical, structure mode of development, etc.) to those seen in mammals, just as the normal fish thyroid is similar to the normal mammalian thyroid. There is however one important minor difference that has figured prominently in the histological interpretation and significance of the active thyroid hyperplasia of certain fish, viz: the brook trout and salmon. This difference is that the fish thyroid is not encapsulated while the thyroids of all animals from amphibia up are enclosed in a definite fibrous membrane. In mammalian goitre it has been established that ordinary thyroid hyperplasia does not penetrate this capsule, but that the capsule and stroma hypertrophy parallel with the epithelial proliferations, so that when the thyroid growth penetrates the capsule and invades surrounding structures, such manifestations are taken as positive signs of malignancy. This same standard cannot be applied to fish thyroids where normally the gland is without any capsule and the alveoli widely distributed in the areolar space around the ventral aorta. By studying a large series it is easily demonstrated that this areolar space is always filled before there is evidence of thyroid growth into bone, muscle, etc. It is also clear that when a gland whose normal follicles are so widely scattered (involving bone and muscle, etc.) undergoes hyperplasia the histological picture would present the appearance of actual invasion of the structures adjacent to which the normal follicles were located. This apparently wild, aimless growth is present as well in the early stages of hyperplasia as in the late stages. The differences are only in the degree and extent of the atrophy and absorption of these contiguous structures as bone, muscle and cartilage. Out of some three hundred and fifty specimens examined from the tanks, representing ages from eight months to two and

two-thirds years, the epithelial growth had involved all the structures beneath the pharyngeal mucosa in every specimen. On the other hand there was not a single instance where the growth had penetrated the skin by actual invasion. The skin over a growing tumor is thinned by pressure atrophy and gradually gives way to ulceration when the actual break in continuity occurs.

Pick has emphasized the fact that there are several histological types of the tumor growth which he has likened to the variations seen in the inoculable breast tumor of mice. One of these types is associated with a great increase in connective tissue and gives the appearance of a scirrhus carcinoma in which the epithelial elements grow in columns and strands, and are not differentiated into actual alveoli. This scirrhus effect is never seen in the early stages of the proliferation, but becomes evident when the growth has extended to the more resistant structures as bone and skin. In all the cases where the epithelial proliferation has extended to the skin, muscle or bone, there is a zone of fibrous overgrowth with the consequent destruction and distortion of the epithelial elements. Aside from this localized and peripheral sclerosis there is also in older fish a less marked general increase in the stroma that becomes evident in the cases undergoing or which have undergone spontaneous involution (reversion) to the colloid state. There are other types which represent all the stages of the (reversion) involution from the marked hyperplasias with no colloid and high columnar epithelium down to quite uniform colloid transformations with cubical epithelium and abundant colloid. Pick has roughly divided these stages of thyroid change into two types which he designates as *hetero* and *homo* types of carcinoma. By "Heterotype" he means those forms in which the evidence of active glandular hyperplasia with reduced colloid and high columnar epithelium predominates and under "Homotype" he includes all forms partaking of the colloid or normal type of gland in which there are well marked accumulations of colloid and the epithelium is cubical.

To one familiar with the cycle of changes that the thyroid follicles undergo in the developing hyperplasia and its involution (reversion) to colloid goitre during the stage of recovery, it is not necessary to introduce these terms to explain the several histological stages of the process. The observed histological phenomena are more clearly explained by comparing them with the similar phenomena of hyperplasia and involution as seen and studied in the mammalian thyroid. (Marine & Lenhart, Johns Hopkins Hosp. Bul. 1909 XX 131.)

Summing up then, the histological evidence of carcinoma is not convincing when one follows the process from its beginning up to the stages of marked hyperplasia with visible tumor formation and bears in mind the fact that the normal gland is widely distributed and *not* encapulated. The terms *homo* and *hetero* types while per-

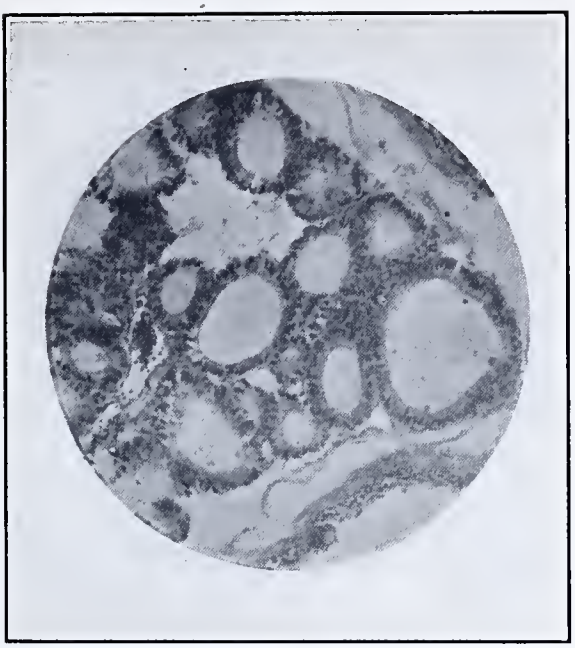

FIG. 7.

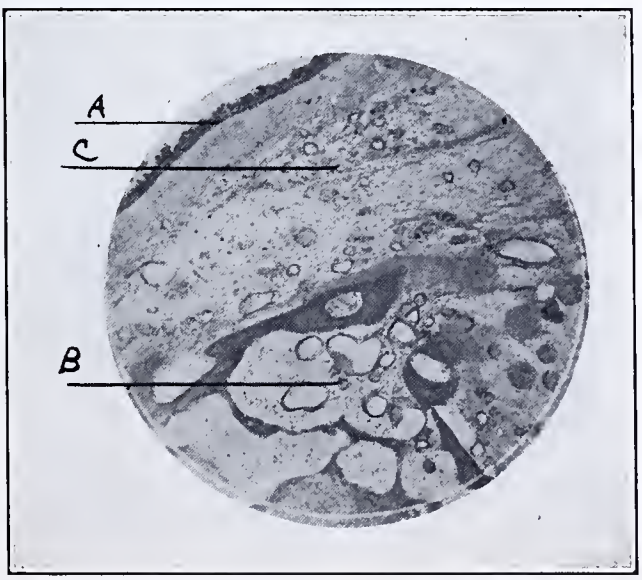

FIG. 8.

fectly justifiable on the bases of microscopic work alone, are not upheld by physiological tests (with iodin) and are more rationally regarded as stages in the cycle of cell changes occurring in the development and involution (reversion) of the hyperplasia.

(c) *Colloid glands* (see figs. *8 and 9*) in fish do not differ in any essential way from similar glands in the higher animals nor do they differ from normal glands in any essential way except in size and in showing the vestiges of previous active hyperplasia. These marks of a previous hyperplasia, however, are well defined and characteristic. The changes are to be found in the size, shape and distribution of the follicles; in the blood vessels and stroma. The distribution of the follicles is that attained during the stage of active hyperplasia and in examples that have been preceded by marked hyperplasia, the rounded follicles are present in bone, muscle and all the tissues beneath the pharyngeal mucosa including the extensions out along the gill arch arteries into the gills. The follicles are everywhere filled with colloid which is normal in appearance and distribution. The epithelium lining the follicles is normal (flat cubical). The stroma is increased, although to this statement there are exceptions depending on the extent of the fibrosis occurring during the period of active hyperplasia and the extent of the absorption which normally occurs during the process of involution (reversion). The blood supply which is so markedly increased in the active hyperplasia is reduced to conform with that of a normal gland. The process is similar to that obtaining in mammals viz: a physiological endarterial thickening. The histological appearance of colloid glands in fish is therefore similar in all respects to colloid glands occurring in mammals whether of spontaneous origin or induced by the action of iodin. The important question is, whether there is a stage in these hyperplasias that will not react with iodin or undergo spontaneous recovery when removed from their unfavorable environment. Experiments are now being made to ascertain if possible to what degree the hyperplasia can go and still remain susceptible to physiological reactions. If a stage in these hyperplasias can be demonstrated when they do not react to iodin or to favorable changes in environment, then a diagnosis of carcinoma would seem justifiable. It would seem possible that such a stage may be found and as pointed out above would be in harmony with our present views of cancer development. It seems to be definitely established that true cancer of the thyroid in dogs and man does not show the characteristic reaction with iodin or to environmental changes.

(d) *Circumscribed Tumor Formations.* (See figs. *10 and 11.)* Pick (loc. cit.) has pointed out the generally symmetrical development of these fish goitres just as is true of all true goitres in all animals.

There may develop, however, in all mammalian goitres rounded or circumscribed masses apart from the main body of the goitre. These are usually called simple adenoma or foetal adenoma, depending on the histological appearance. Now and then one sees in these fish goitres similar rounded and symmetrical circumscribed growths which histologically may be as fully differentiated as the surrounding ordinary hyperplasia, but more commonly are less well differentiated, as evidenced by the lack of regular uniform types of cell, or the more marked infolding and piling up of epithelial elements and lessened stroma—all these factors being interpreted as evidence of a more rapid growth and a greater physiological independence than the ordinary surrounding hyperplasia. Whether these more independent growths in fish goitres react with iodin or not has not been ascertained, as it was not our fortune to have included any such specimens in the iodin experiment. As is well known in mammals these growths do not react either with iodin or spontaneously in any constant manner, although they do in most cases return to the resting state after variable periods of growth while occasionally they continue their growth with manifestations of carcinoma. The circumstances that determine continuation or cessation of growth are unknown to us and are quite beyond any known method of physiological control This is in sharp contrast with the simple goitrous hyperplasias which are under definite physiological control.

DISCUSSION.

The first question to be considered is the nature of this disease. Is it true carcinoma or is it only ordinary goitre? Up to the present time this disease has been considered carcinoma. This opinion has been arrived at as a result of histological examinations. The essential feature here being that it invades bone, muscle, cartilage, etc. Such findings in mammalian tissues would be strong evidence of malignancy, but it happens that this standard cannot be applied to fish thyroid for the simple reason that the gland is normally widely and loosely distributed and without a capsule, so that *any* growth of the gland would give the appearance of invasion of the surrounding tissues. The error of depending too much on the histological appearance in mammalian thyroid changes has been frequently emphasized and from our rather extensive experience with mammalian thyroid changes we have learned to be very careful in diagnosing thyroid carcinoma. The reason is that the thyroid undergoes such rapid changes that very unusual pictures are produced both of cell proliferation and of cell degeneration in thyroids that react with iodin. Our iodin experiments have shown that these fish hyperplasias react with iodin and any thyroid hyperplasia that reacts with iodin we believe should not be considered cancer. We have

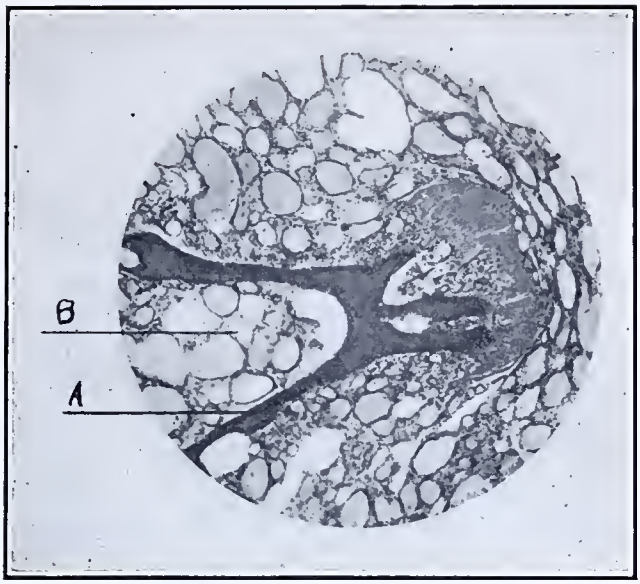

FIG. 9.

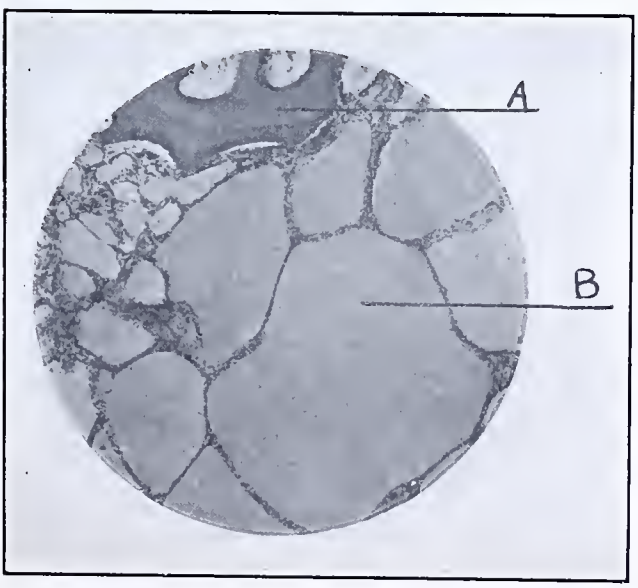

FIG. 10.

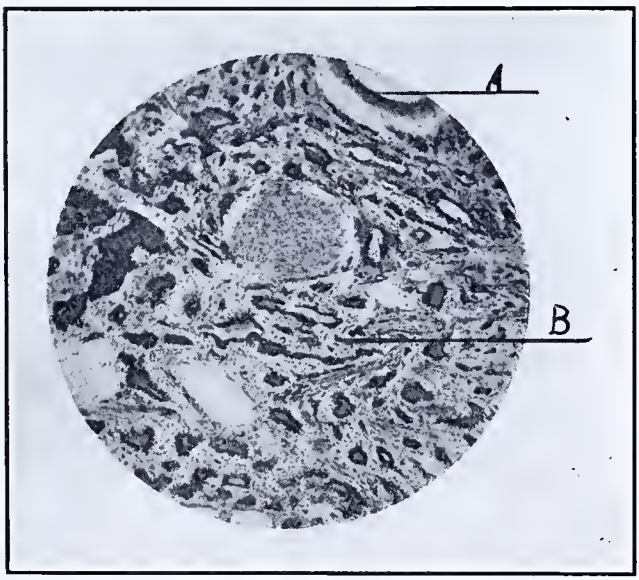

FIG. 11.

never seen true carcinoma, whether in man or dogs, react with iodin or undergo spontaneous recovery. This fish disease does both. If one merely takes the fish from these tanks and puts them in the brook their thyroids in the course of three to four months will return to the quiescent or colloid state. In the light of these findings we look upon the disease as ordinary endemic goitre, at least in the stages it is ordinarily seen and studied.

The use of the word "epidemic" is misleading in connection with goitre inasmuch as neither infection nor contagion have ever been demonstrated. The term unfortunately was introduced before the days of bacteriology to explain the rather rapid development of numbers of cases of goitre among soldiers confined in barracks in goitrous districts.

As to the cause of this disease the same views are held as in mammalian goitre. These may be divided into two groups (1) *Infectious*, (2) *Metabolic*.

Taking up the infectious theory, there are abundant grounds for such a possibility when one considers the flora of the tanks in which the fish are kept. Here are to be found many varieties of fungi, algae, bacteria, amoebae, gregarines, diatomes, worms, etc. Hofer, Bonnet and Pick have all made extremely careful histological examinations for the presence of parasites, particularly sporozoa, with uniformly negative results. It is impossible to exclude infection at the present state of our knowledge in any form of goitre and particular in the disease in fish where conditions are ideal for the rapid development of large numbers of cases. It is certain that it does not obey the laws of known water borne infections for the reason that fish placed in the tail-race immediately below the last pair of tanks are able to recover from any hyperplasia they had at the time of putting them there or if their thyroids were normal at the time of putting them there they were able to maintain normal glands even though living in the theoretically most polluted and infected water.

In considering the second or metabolic theory there are three factors of major importance, viz: *over feeding, over crowding* and *diminished water supply*. (a) Over feeding—The food consists of hashed liver, heart and lung tissue usually of hogs or sheep, fed in the raw state. It is impossible to prevent a certain amount of decomposition taking place before feeding this material. The fish are fed one or more times daily at which times they are given all they can eat and in all feedings a certain amount goes to the bottom uneaten where it undergoes further decomposition into simpler and more soluble compounds. (a) Overcrowding of the fish is extremely common. We have seen as high as five to seven hundred two year old fish in a tank 14 by 6 by 3 feet where the water intake was only sufficient to replace the contents once every four to six hours.

The effect of overcrowding is to increase the amount of decomposing food on the bottom, to increase the amount and concentration of feces and urine which settle to the bottom to a large extent. Here extensive bacterial decomposition is continually going on by means of which the soluble parts are carried away. Under such conditions the extent of water pollution would vary greatly with the amount of water passing through. The question of oxygen supply is also of importance. There are no signs of acute asphyxiation as would be indicated by the fish coming to the surface but in such a laboratory of chemical decomposition products the question of diversion of part of the oxygen would have to be considered. The increased respiratory rate, the swollen and inflamed condition of the gill filaments would suggest the presence of some toxic agent or the absence of some substance normally needed to neutralize their injurious effects.

(c) Diminished water supply especially during the summer months when water is most needed to take away the increased decomposition products of the excess of food usually given during this season of the years, is a factor of greatest importance. Then too, fish are more active during the summer months and more fecal and urinary products are deposited. Tumor growth likewise occurs more rapidly during these months. These three factors of *food, crowding* and *limited water supply* together with the various possibilities and minor factors into which they are divisible, we believe include the cause of this disease. It is not likely that a single substance excites the thyroid reaction, but rather that the thyroid reaction is a result of the activity of a great variety acting together. Then again these substances may not act as direct excitants but through the respiratory or other systems in an indirect manner, by influencing the gaseous interchanges, the oxidation process in the body, etc.

CLINICAL MANIFESTATIONS OF GOITRE IN FISH.

We could make out no conditions comparable to cancer cachexia seen in higher animals. The fish on the contrary are abnormally large for a given age, excessively fat and, in contrast with their fellows living wild in the streams, are *weak, sluggish, lumbersome* creatures with little resistance when taken by hook and line. They also die more quickly than normal fish following their removal from the water Plehn (loc. cit.) thought she could make out evidences of cancer cachexia. Death we believe rarely results directly from the disease but through intercurrent infectious processes particularly gill infections, fungus, etc. The major direct effect of the tumor seems to be mechanical. Thus the locking of the gills prevents or lessens normal respiratory movements and ability to feed. As to the duration of life in these affected fish we have no complete observations,

Smith (Washington Med. Annals 1909 VIII 313) has lately recorded his observations as to the effect of goitre in fish and our observations above recorded are in harmony with them. He also adds that the affection does not seem to shorten the life of the fish to any appreciable extent. In general this is true of the thyroid effect *per se* but the affected fish are certainly more susceptible to intercurrent disease and it indirectly shortens life.

Finally some mention should be made of associated changes in these goitrous fish. We have already mentioned the fact that these fish are large for their age, excessively fat and as a rule show excessive skin pigment, and even in the thyroid and internal organs there may be pigment development. There is also constant *splenic* and *lymphoid enlargement* just as is so often seen in mammalian goitre. The question of the blood condition has not been investigated.

When we take into consideration all observed factors and compare them with developing goitre in mammals and see so many factors in common with ordinary goitre and but one (the histological evidence of invasion) contrary, there does not seem to be any sound basis for considering this disease other than ordinary endemic goitre.

METHODS OF PREVENTION AND CURE.

These are simple in spite of the fact that the cause is unknown. They consist in reproducing natural conditions as far as possible. The experiment of placing some of these goitrous fish in the open brook and noting the complete return of the thyroid hyperplasia to the colloid or quiescent state includes all the essentials in the prevention or cure of this disease.

In the analysis of the above experiment the following points may be emphasized:

(1) The food should be reduced so that none is left to decompose on the bottom of the ponds.

(2) The number of fish should conform with the water supply.

(3) The water supply should be sufficient to take away the excreta or at least dilute it since the development of this disease varies with the concentration of the chemical decomposition products of food and excreta.

Experiments with boiling the food and with changing the proportions of fats, carbohydrates and proteins have not progressed sufficiently to say what influence these may have on the development of the disease.

Another method which is satisfactory in laboratory experiments, is the administration of iodin containing substances. Their administration stops the further development of hyperplasia and causes involution (reversion) to the colloid state. Whether such a remedy

is practical from a commercial or economic standpoint remains to be tried. Of these two methods the first or natural one should, in our judgment, be instituted wherever possible.

CONCLUSIONS.

(1) That the socalled carcinoma of the thyroid of brook trout is, in its early stages at least, nothing more than severe endemic goitre. The possibility that this physiological hyperplasia may go over into actual carcinoma is not denied, and is in harmony with the more modern views of cancer development.

(2) *Overfeeding, overcrowding* and a *limited water supply* are the three major factors in the production of filthy, unhygienic tanks or ponds and these unsanitary, unhygienic and filthy tanks are in a very important but still unknown manner associated with the development of thyroid hyperplasia.

(3) Prevention and cure is favored by adjusting the amount of food and the number of fish to the water supply or by the addition of iodin containing substances to the water passing through the ponds.

NOTE.—This study was made possible through facilities granted by and through the co-operation of the Honorable W. E. Meehan, Commissioner of Fisheries for the State of Pennsylvania. We wish to express our obligations and thanks to Mr. Meehan and also to the officers of the private club at whose hatchery our experiments and observations were made.

EXPLANATION OF FIGURES.

Fig. 1. Drawing illustrating the location, size and relations of an external visible goitre of the brook trout. Note also the swollen and hypertrophied appearance of the gills—socalled "soregill." Note also the great increase in skin pigment and absence of the red and yellow color spots.

Fig. 2. Semi-diagramatic drawing of the *plan*, *arrangements* and *location* of the *Hatchery*, *water supplies*, *houses* and *retaining tanks*.

Fig. 3. Drawing illustrating the location of the thyroid area in the bony fish (Teleostei.)

A.—Thyroid area.
B.—1st gill segment.
C.—2d gill segment.
D.—3d gill segment.
E.—4th gill segment.

Fig. 4. Drawing illustrating the distribution of the normal thyroid follicles in the brook trout as seen in a transverse section of the thyroid area at the level of the 2d gill segment.

A.—Pharyngeal mucosa.
B.—Thyroid follicles.

Fig. 5. Microphotograph of the normal trout thyroid illustrating the absence of a capsule, the scattered arrangement of the follicles and their relation to striped muscle, bone and cartilage.

A.—Thyroid follicles.
B.—Striped muscle.
C.—Bone.
D.—Cartilage.

Fig. 6. Microphotograph of active thyroid hyperplasia showing:

A.—Invation of striped muscle.
B.—Relation to bone.

Fig. 7. Microphotograph of active hyperplasia showing the regular, uniform arrangement and high columnar type of the follicular epithelium and absence of colloid.

Fig. 8. Microphotograph of colloid goitre from a 3 2-3 years old fish from the trout stream illustrating spontaneous recovery notwithstanding the invasion of bone and subpharyngeal tissues.

A.—Pharyngeal mucosa.
B.—Typical colloid follicles in bony angles.
C.—Typical colloid follicles in the subpharyngeal tissue.

Fig. 9. Microphotograph illustrating the effect of iodine on the marked active hyperplasias. Note that all follicles are lined with flat, cubical epithelium; that all follicles contain colloid; that the thyroid tissue has invaded all the structures beneath the pharyngeal mucosa.

A.—Bone.
B.—Thyroid follicles in bone that have completely reverted to the colloid or quiescent state.

Fig. 10. Microphotograph of a large external goitre illustrating relation of follicles to bone.

A.—Bone.

B.—Large colloid follicles of the reverted thyroid hyperplasia.

Fig. 11. Microphotograph of a large external goitre illustrating the atrophy and degeneration of the follicular apithelium with the extreme fibrosis giving the appearance of scirrhus carcinoma. This is exactly similar to the cretinoid or myxodematous atrophy in mammals.

A.—Artery.

B.—Distorted follicles in the fibrous stroma overgrowth.

TABLE I—TANK (POND) SERIES.

House No.	Tank (Pond) No.	Age of Fisb.	Histological Condition of Thyroid.			Classification.
			Stainable Colloid.	Distribution, Infiltration, etc.	Epithelium.	
Normal from brook above all houses,		2⅔ years,	Normal,	Scattered groups of follicles about aorta and isolated follicles throughout areolar tissue.	Regular, flat cubical,	Normal thyroid.
Normal from brook above all houses, male.		1⅔ years,	Normal,	Scattered groups of follicles about aorta and isolated follicles throughout areolar tissue.	Regular, flat cubical,	Normal tbyroid.
I	1	⅔ years,	Absent,	Entire thyroid area filled, invasion of bone and muscle.	Uniform, high columnar,	Marked glandular hyperplasia.
I	2	⅔ years,	Nearly absent,	Entire thyroid area filled, invasion of bone and muscle with extension into gill segments.	Uniform, high columnar,	Marked glandular byperplasia.
I	3	⅔ years,	Present, reduced,	Entire space about aorta filled with thyroid tissue, bone and muscle not involved.	Uniform, columnar,	Early moderate glandular hyperplasia.
I	4	⅔ years,	Reduced,	All structures beneath pbaryngeal mucosa, including bone and muscle, involved.	Uniform, columnar,	Moderate glandular hyperplasia.
I	5	⅔ years,	Nearly absent,	All structures beneath pharyngeal mucosa, including bone and muscle, involved.	Uniform, columnar,	Moderate glandular hyperplasia.
II	6	⅔ years,	Nearly absent,	Entire areolar space about aorta filled.	Uniform, columnar,	Moderate glandular hyperplasia.
III	7	⅔ years,	Absent,	Throughout all structures beneath pharyngeal mucosa infiltration and atrophy of bone, muscle, etc.	Uniform, high columnar,	Marked glandular byperplasia.
III	8	⅔ years,	Reduced,	Entire areolar space about aorta filled.	Uniform columnar,	Moderate glandular hyperplasia.
III	9	⅔ years,	None,	Entire space beneath pharyngeal mucora occupied destruction of bone and muscle.	Uniform, high columnar,	Marked glandular byperplasia.
III	10	⅔ years,	None,	Entire space beneatb pharyngeal mucora occupied destruction of bone and muscle.	Uniform, high columnar,	Marked glandular hyperplasia.

TABLE 1—Continued.

House No.	Tank (Pond) No.	Age of Fish.	Histological Condition of Thyroid.			Classification.
			Stainable Colloid.	Distribution, Infiltration, etc.	Epithelium.	
IV	11	⅔ years,	Nearly absent, ----------	Entire space beneath pharyngeal mucora occupied destruction of bone and muscle.	Uniform, high columnar,-	Marked glandular hyperplasia.
IV	12	⅔ years,	Absent, ----------	Entire space beneath pharyngeal mucora occupied destruction of bone and muscle.	Uniform, high columnar,-	Marked glandular hyperplasia.
IV	13	⅔ years,	Absent, ----------	Entire space beneath pharyngeal mucora occupied destruction of bone and muscle.	Uniform, high columnar,-	Marked glandular hyperplasia.
V	14	1⅔ years,	Absent, ----------	Entire sub-pharyngeal space filled, cartilage, bone and muscle destroyed extension into gills.	Uniform, high columnar,-	Marked glandular hyperplasia.
V	15	1⅔ years,	Absent, ----------	Entire sub-pharyngeal space filled, cartilage, bone and muscle destroyed extension into gills.	Uniform, high columnar,-	Marked glandular hyperplasia.
V	16	1⅔ years,	Absent, ----------	Entire sub-pharyngeal space filled, cartilage, bone and muscle destroyed extension into gills.	Uniform, high columnar,-	Marked glandular hyperplasia.
VI	17	1⅔ years,	Absent, ----------	Entire sub-pharyngeal space filled, cartilage, bone and muscle destroyed extension into gills with marked fibrosis.	Uniform, high columnar,-	Marked glandular hyperplasia.
VI	18	1⅔ years,	Absent, ----------	Entire space beneath pharyngeal mucosa filled, bone, cartilage and muscle eroded.	Uniform, high columnar,-	Marked glandular hyperplasia.
VI	19	1⅔ years,	Absent, ----------	Entire space beneath pharyngeal mucosa filled, bone, cartilage and muscle eroded.	Uniform, high columnar,-	Marked glandular hyperplasia.
VII	20	1⅔ years,	Reduced but everywhere present in the follicles,	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform, columnar, ------	Colloid—moderate marked glandular hyperplasia, involuting (reverting).
VII	21	1⅔ years,	Nearly normal, ----------	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform high cubical, ----	Colloid—early glandular hyperplasia, involuting (reverting).

VII	22	1⅔ years,	Present throughout, reduced.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform, columnar, ------	Colloid—moderate glandular hyperplasia, involuting (reverting).
VIII	23	1⅔ years,	Present throughout, reduced.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform, columnar, ------	Colloid—Hoderate glandular hyperplasia, involuting (reverting).
VIII	24	1⅔ years,	Present throughout, reduced.	Entire spaee beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform, columnar, ------	Colloid—Hoderate glandular hyperplasia, involuting (reverting).
VIII	25	1⅔ years,	Present throughout, reduced.	Entire space beneath pharyngeal mucosa filled with thyroid, infiltration of bone and muscle.	Uniform, columnar, ------	Colloid—Hoderate glandular hyperplasia, involuting (reverting).
IX	26	2⅔ years,	Absent, ----------	Every structure beneath pharyngeal mucosa infiltrated, extension into gills.	High columnar, slightly irregular.	Marked glandular hyperplasia.
IX	27	2⅔ years,	Absent, ----------	Every strueture beneath pharyngeal mucosa infiltrated, extension into gills.	High columnar, slightly irregular.	Marked glandular hyperplasia.
IX	28	2⅔ years,	Present, reduced, --------	Every structure beneath pharyngeal mucosa infiltrated, extension into gills.	High columnar, ----------	Marked glandular hyperplasia.
IX	29	2⅔ years,	Absent, ----------	Follicles in many places undifferentiated.	High columnar, ----------	Marked glandular hyperplasia with extreme generalized fibrosis of the thyroid area.
X	30	2⅔ years,	Reduced, ----------	Follicles in many places undifferentiated.	Irregular columnar, ------	Colloid—marked glandular hyperplasia.
X	31	2⅔ years,	Reduced, ----------	All strueture invaded including extension to gills. Extreme general fibrosis.	Irregular columnar, ------	Colloid—marked glandular hyperplasia.
X	32	2⅔ years,	Reduced, ----------	All structures invaded including extension to gills. Extreme general fibrosis.	Irregular columnar, ------	Colloid—moderate glandular hyperplasia, extreme general fibrosis.
X	33	2⅔ years,	Present everywhere, ------	All structures invaded including extension to gills. Extreme general fibrosis.	Irregular columnar, ------	Colloid—moderate glandular hyperplasia, generalized fibrosis of gland area.
XI	34	2⅔ years,	Reduced, ----------	All structures invaded including extension to gills. Extreme general fibrosis.	Irregular columnar, ------	Colloid—marked glandular hyperplasia, extensive generalized fibrosis of gland area.
XI	35	2⅔ years,	Reduced, ----------	All structures invaded including extension to gills. Extreme general fibrosis.	High columnar, ----------	Marked glandular hyperplasia, slight general fibrosis.
XI	36	1⅔ years,	Reduced, ----------	All sub-pharyngeal structures involved including bone, muscle and extension to gills.	High columnar, ----------	Marked glandular hyperplasia.
Female from raceway below all houses.		3⅔ years,	Normal, ----------	All sub-pharyngeal structures involved including bone, muscle and extension to gills.	Low cubical, ----------	Pure colloid goitre, transfered from tank to brook as a three years old in April, 1909, spontaneous recovery.

TABLE 1—Continued.

Tank	(Pond) No.	Age of Fish.	Histological Condition of Thyroid.			Classification.
			Stainable Colloid.	Distribution, Infiltration. Etc.	Epithelium.	
From raceway below all houses.		1⅔	Normal,	All tissues beneath pharyngeal mucosa infiltrated including bone and muscle.	Flat cubical,	Pure colloid goitre (transferred from tank to brook as one year old in spring, 1909, spontaneous recovery.
From raceway below all houses.		⅔	Present,	Perhaps a very slight increase in the number of thyroid follicles, practically normal, no invasion of bone or muscle.	Cubical,	Normal (normal-early glandular hyperplasia).
From trout stream.		3⅔	Normal,	All structures beneath pharyngeal mucosa infiltrated—bone, muscle and extension to gills.	Flat cubical,	Pure colloid goitre (this is an example of the three years old fish transferred from tanks to trout stream in April, 1909, spontaneous recovery.

TABLE 3—EFFECT OF ADMINISTRATION OF IODIN.

Number of fish.	Date spec. taken.	Iodin administration—days.	Age.	Histological Condition of Thyroid.			Classification.
				Stainable colloid.	Distribution, Infiltration, Etc.	Epithelium.	
1 male,	Oct. 11,	2 days,	1⅔ years,	Present throughout, but very scanty.	Throughout all the structures beneath the pharyngeal mucosa including bone, muscle, etc.	Columnar,	Marked glandular hyperplasia.
2 male,	Oct. 13,	4 days,	1⅔ years,	Present throughout, but very scanty.	Throughout all the structures beneath the pharyngeal mucosa including bone, musele, etc.	Columnar,	Marked glandular hyperplasia.
3 female,	Oct. 15,	6 days,	1⅔ years,	Present throughout, reduced.	Throughout all the structures beneath the pharyngeal mucosa including bone and musele.	Columnar,	Marked glandular hyperplasia.
4 female,	Oct. 17,	8 days,	1⅔ years,	Present throughout, reduced.	Throughout all the structures beneath the pharyngeal mucosa ineluding bone and muscle.	High cubical,	Colloid — moderate marked glandular hyperplasia involuting reverting).
5 male,	Oct. 19,	10 days,	1⅔ years,	Present throughout, sligbtly redueed.	Througbout all tbe structures beneath the pharyngeal mucosa ineluding bone and muscle.	Higb cubical,	Colloid — moderate glandular byperplasia involuting (revertting).
6 male,	Oct. 21,	12 days,	1⅔ years,	Present in all follielcs, slightly redueed.	Throughout all the structures beneath the pharyngeal mucosa ineluding bone and musele.	Low eolumnar,	Colloid — moderate glandular hyperplasia involuting (revertting).
7 female,	Oct. 23,	14 days,	1⅔ years,	Present in all follicles, nearly normal.	Throughout all the struetures beneath the pharyngeal mucosa ineluding bone and musele.	Cubical,	Colloid— early moderate glandular hyperplasia involuting (reverting).
8 female,	Oct. 25,	16 days,	1⅔ years,	Normal throughout,	Throughout all the tissues beneath the pharyngeal mucosa ineluding bone, muscle and extensions into gill segments.	Low cubical,	Colloid—early glandular hyperplasia involuting (reverting).

TABLE 3—Continued.

No. of fish.	Date spec. taken.	Iodin administration-days.	Age.	Histological Condition of Thyroid.			Classification.
				Stainable Colloid.	Distribution, Infiltration, Etc.	Epithelium.	
9 female, ------	Oct. 27,	18 days,--	1⅔ years, ---	Normal throughout,	Throughout all tissues beneath the pharyngeal mucosa including bone, muscle and cartilage erosions.	Low cubical, -----	Colloid—early glandular hyperplasia involuting (reverting).
10 male, -------	Oct. 29,	20 days,--	1⅔ years, ---	Normal throughout,	Throughout all tissues beneath the pharyugeal mucosa including bone, muscle aud cartilage erosions.	Low cubical, -----	Pure colloid goitre complete involution (reversion).
11 male, --------	Oct. 31,	22 days,--	1⅔ years, ---	Present in all follicles, perhaps slightly less than normal.	Throughout all tissues beneath the pharyngeal mucosa including bone, muscle and cartilage erosions.	Cubical, ----------	Colloid—early glandular hyperplasia involuting (reverting).
12 male, --------	Oct. 31,	22 days,--	1⅔ years, ---	Abundant in all follicles.	Throughout all tissues beneath the pharyngeal mucosa includiug bone, muscle and cartilage erosious.	Flat cubical, ------	Pure colloid goitre complete involution (reversion).
13 female, ------	Oct. 31,	22 days,--	1⅔ years, ---	Abundant in all follicles.	Throughout all tissues beneath the pharyngeal mucosa including boue, muscle aud cartilage erosions.	Low cubical, ------	Colloid—early glandular hyperplasia, very pronounced fibrous stroma, involutiug (reverting).
14 female, ------	Oct. 31,	22 days,--	1⅔ years, ---	Abundant in all follicles.	Throughout all tissues beneath the pharyngeal mucosa including bone, muscle and cartilage erosions.	Low cubical, ------	Pure colloid goitre, complete involutiou (reversion).
15 male, ------	Oct. 31,	22 days,--	1⅔ years, ---	Abundant in all follicles.	Throughout all the tissues beneath the pharyngeal mucosa including boue, muscle and extensions into the gill segments.	Low cubical, ------	Pure colloid goitre, complete involution (reversion).